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Membrane composition and ion-permeability in extremophiles

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Abstract

Protons and sodium ions are the only used coupling ions in energy transduction in Bacteria and Archaea. At their growth temperature, the permeability of the cytoplasmic membrane of thermophilic bacteria to protons is high as compared to sodium ions. In some thermophiles, therefore, sodium is the sole energy coupling ion. Comparison of the proton- and sodium permeability of the membranes of variety of bacterial and archaeal species that differ in their optimal growth temperature reveals that the permeation processes of protons and sodium ions must occur by different mechanisms. The proton permeability increases with the temperature, and has a comparable value for most species at their respective growth temperatures. The sodium permeability is lower than the proton permeability and increases also with the temperature, but is lipid independent. Therefore, it appears that for most bacteria the physical properties of the cytoplasmic membrane are optimised to ensure a low proton permeability at the respective growth temperature.

Keywords: Membrane; Thermophile; Psychrophile; Alkaliphile; Acidophile; Permeability

Contents

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1. Introduction

Biological cells use membranes to organise space into different compartments [1]. The barrier function of these membranes is of fundamental importance in both the structural organisation and function of all prokaryotic and eukaryotic cells. In bacteria, the cytoplasmic membrane divides the cell in two compartments, i.e., the cytoplasm and the outside of the cell. In the absence of such a distinct barrier, all kinds of molecules would freely move between the inside and the outside of the cell, and stable, self-regulating systems would become impossible. On the other hand, when the barrier function would be too high, molecular movement across the membrane would become very difficult, and cells would not be able to change their composition in response to changes in the environment. Obviously, life could not be sustained in this case either. Thus, although the lipid membrane behaves as a barrier, at the same time the permeation of molecules across the membrane is of vital importance.

During evolution, nature has developed many different ways of permeation, depending on the nature of the permeating molecule. In the case of very large, hydrophilic or charged molecules, the lipid bilayer itself presents too much resistance, and special mechanisms are required to allow the membrane permeation of such molecules. These mechanisms being either active or passive, may for instance involve transport proteins that reduce the barrier locally in the membrane, or carrier molecules that permeate themselves at a significant rate and can take another molecule along. Many smaller lipophilic molecules, however, are able to permeate across the membrane without any specific transport mechanism. This is called the basal permeation process or passive diffusion.

On the molecular level, biomembranes are quite complex. The basic structural element is a bilayer of lipid molecules which serves as a two-dimensional solvent for various proteins, as conceived by the fluid-mosaic model [2]. A question that recurs frequently concerns the large diversity of lipids found in biological membranes. When the different polar head groups and acyl chains are taken into account, a given membrane may contain well over 100 unique species. Does each lipid have a specific functional role or is the diversity of lipids just a manifestation of accidental evolutionary development? Some functions are immediately obvious in a qualitative sense in terms of optimisation of physical properties such as membrane fluidity, lipid polymorphism, and other physicochemical attributes [3].

Questions related to lipid diversity and membrane barrier function are especially intriguing when one considers the bacteria and archaea that belong to the group of extremophiles [4,5]. Some of these organisms are able to grow at temperatures below 0°C or at up to 110°C, at pH values below 0.5 or up to 12.0, at very high salt concentration, i.e., above 3.5 M NaCl, or under high pressure of up to 1100 atmosphere. To allow efficient energy transduction, these organisms must have developed mechanisms to maintain the barrier function of the cytoplasmic membrane when growing in their respective nice. In addition to mechanisms needed to stabilize proteins, an extreme form of optimisation of the physical properties of the membrane is needed in order to cope with these hostile environments.

2. Microbial membrane organisation

The cytoplasmic membrane of most bacteria consist of a lipid bilayer. The core of these lipids contain two straight-chain fatty acids ester-linked to a glycerol moiety (Fig. 1) [6]. The structure of the membrane depends largely on hydrophobic effects (which also control protein structures). The repulsion of the lipid hydro-carbon chains by water drives the chains into an environment free from water. The amphipatic

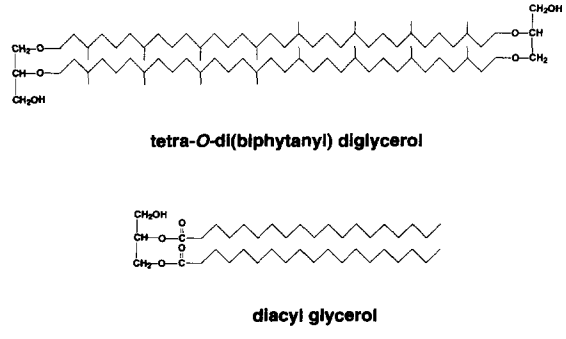

Fig. 1. Backbone structures of the archaeal tetra-ether lipids and bacterial glycerol-ester lipids.

nature of the polar membrane lipids directly defines the bilayer structure. It provides a hydrophobic environment in the middle of the bilayer for hydrocarbon chains, with the lipid polar head groups sticking into the aqueous phase. Dynamically, the lipid bilayer is highly anisotropic; the interior of a bilayer is mostly ordered, and only a small region in the middle is liquid-like [7]. The cytoplasmic membrane is at the optimum growth temperature in the liquid-crystalline phase [8], but other phases can also be found, depending on a variety of factors such as temperature, fatty acid composition, pH and the presence of divalent cations [9,10]. An important parameter in the physico-chemical behaviour of the membrane is the temperature, T_c, around which the transition between gel to liquid-crystalline states takes place. With the appreciation of the importance of membrane fluidity, it soon became clear that many organisms actually adjust their lipid composition in response to changes in environmental parameters such as temperature in order to preserve membrane fluidity. For instance, psychrophilic organisms that grow at temperatures ranging from just below the freezing point up to 30°C, maintain the fluidity of the membrane at the low temperatures by including a preponderance of unsaturated short (C_{14}-C_{16}) acyl chains in their lipids [9]. The unsaturated acyl chains have a much lower melting temperature than those containing saturated chains. Many organisms systematically increase (*or decrease*) the concentration of unsaturated relative to saturated chains in their lipids when the growth temperature is decreased (*or increased*). Likewise, the vast majority of the acyl chains of the lipids of thermophilic bacteria are long (C_{18}–C_{24}) and saturated. Additional rigidity can be obtained by the inclusion of branched or cyclic acyl chains, hopanoids (cholesterol-like molecules) [11], or in some cases, saturated, membrane-spanning phospholipids. The ability of bacteria to maintain their membrane in a liquid-crystalline state through variation of the lipid composition when subjected to temperature or other environmental changes has been termed 'homeoviscous adaptation' [7]. It is likely that many other examples of lipid diversity (as for instance *lipid polymorphism* [12]) have a function in maintaining the physical properties of the membrane.

Archaea have only recently been identified as a separate biological kingdom [13] and the physico-chemical properties of their membranes have been studied less completely than those of bacteria [14]. During the evolution, Archaea have maintained the integrity and form of the cell by types of cell walls, so-called S-layers [15], and the evolution of unique classes of archaeal lipids [14,16]. The latter may have been developed in response to the extreme conditions of salt concentration, temperature and pressure (e.g., as in deep sea hot springs) under which certain archaea occupy their special niche. Unlike the straight-chain fatty acids and fatty acid ester-linked glycerol lipids that are characteristic of bacteria and eukarya, archaeal lipids are distinguished by isoprenoid and hydroisoprenoid hydrocarbon and isopranyl glycerol ether-linked lipids (Fig. 1) [16,17]. Glycerol ether lipids are also found in other types of cells in minor amounts, but only in archaea have they evolved as the fundamental glycerolipid component. While some ether lipids are structurally analogous to their counterparts in bacteria and eukarya, in particular di-ether lipids, others such as the tetra-ether lipids are not. Most are about forty carbons long and have two polar ends. Such lipids would quite naturally span the lipid bilayer structure and this may well be their configuration in biological membranes. The monolayer organisation of the tetra-ether lipids provides extreme rigidity to these membranes [18]. Further rigidity is provided by the methyl side groups at the branch points in the biphytanyl chains or obtained by the inclusion of cyclopentane rings in the acyl chains [16,17]. In the tetraether lipids of *Sulfolobus solfataricus* and *Thermoplasma acidophilum*, archaea that can grow at temperatures of up to 85°C and a pH of 1–3, the degree of cyclization of the biphytanyl components increases with increasing growth temperature. Although the concept of 'homovisceous adaptation' is not so well developed in archaea, it is unquestionable that these organisms have retained the ability to adjust the lipid composition to environmental alterations.

3. Temperature dependency of proton and sodium transport across bacterial membranes

The presence of a proton gradient across the membrane is of vital importance to the functioning

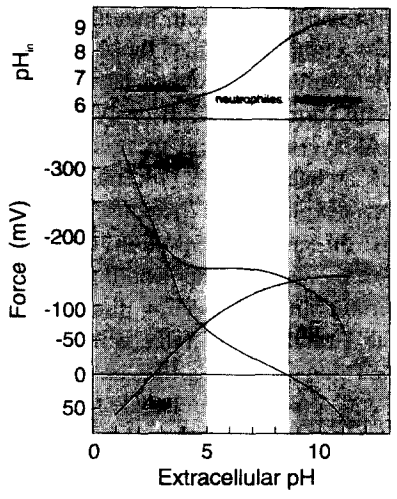

Fig. 2. Schematic representation of the magnitude and composition of the electrochemical gradient of protons ($\Delta\tilde{\mu}_{H^+}$) and intracellular pH as a function of the extracellular pH for acido-, neutro- and alkaliphilic bacteria. The composition of the $\Delta\tilde{\mu}_{H^+}$, i.e., the transmembrane electrical potential ($\Delta\psi$) and pH gradient ($-Z\Delta$pH) are indicated separately.

of bacterial cells [19]. For instance, the electrochemical gradient of protons ($\Delta\tilde{\mu}_{H^+}$) functions as energy source to drive uphill transport of solutes, the synthesis of ATP from ADP and inorganic phosphate, and flagellar motor to provide the organism with motility. By regulating the magnitude of the proton gradient (ΔpH) cells have the ability to maintain their intracellular pH in response to changes in the medium pH. This is in particularly important for bacteria that grow at the borders of the pH ranges, i.e., acidic or alkaline pH values, irrespective of the growth temperature (Fig. 2) [20]. This broad pH range in which bacteria can grow is only possible by the intracellular pH homeostasis. This pH homeostasis can maintain the internal pH in a viable range if the membrane faces a low, but significant level of basal proton permeation but might break down if the proton permeability becomes more prominent under more hostile growth conditions.

The proton gradient across the cytoplasmic membrane is maintain by primary transport systems such as electron-transfer systems and ATPases [21]. These systems translocate protons from the cytoplasm to the external medium and thereby generate a $\Delta\tilde{\mu}_{H^+}$. Alternatively, systems exist that translocate sodium ions across the membrane to generate an electrochemical gradient of sodium ions ($\Delta\tilde{\mu}_{Na^+}$) either through primary expulsion of sodium ions [22], or via secondary mechanisms that involve exchange between protons and sodium ions [23]. Protons and sodium ions are the preferred energy transducing coupling ions used by bacteria and archaea, and obviously, this will only be possible when the membrane has a limited permeability for these ions. Thus, in order to allow efficient energy transduction to take place, the membrane must be tightly sealed for protons and/or sodium ions at the respective growth conditions.

The simplest model system for a biomembrane is provided by the lipid bilayer without proteins [24]. Such a bilayer can be prepared in several ways and can then be studied by physical methods. When dissolved in water, (phospho-)lipids will selfassemble into lipid bilayers and form closed vesicles termed 'liposomes'. These liposomes enclosed an aqueous compartment as in biomembranes. When prepared from natural lipid sources, liposomes present an excellent model system to investigate the permeability properties of bacterial membranes. In this respect, for non-electrolytes it has been shown that the passive permeability characteristics of a biomembrane are essentially the same as those of liposomes prepared from extracted lipids [25]. In a recent study we compared the proton and sodium permeation rates of liposomes composed of lipids derived from various bacteria and archaea in order to categorise the impact of a changing lipid composition on the general permeability properties of the membrane to the two, most important energy transducing ions [26,27]. In this study, entirely unrelated organisms were chosen such that nearly the entire range of temperatures at which these organisms can grow was covered. This included the psychrophilic bacterium *Psychrobacter* sp. (T_{growth} 21°C), the mesophilic Archaeon *Methanosarcina barkeri* (T_{growth} 35°C) and the bacterium *Escherichia coli* (T_{growth} 37°C), the thermophilic bacteria *Bacillus stearothermophilus* (T_{growth} 60°C) and *Thermotoga maritima* (T_{growth} 80°C), and the thermoacidophilic Archaeon *Sulfolobus acidocaldarius* (T_{growth} 80°C).

Comparison of the proton permeabilities (k_{H^+}, first order rate constant for proton permeation) of liposomes composed of lipids derived from a vast

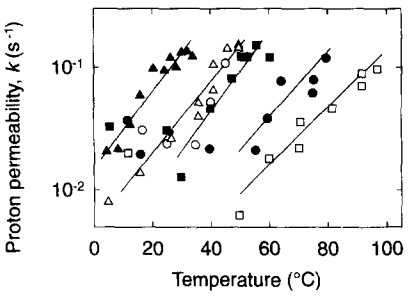

Fig. 3. Temperature dependency of the first order rate constant for the proton permeability (k_{H^+}) of liposomes derived from various bacteria and archaea. Liposomes were composed of lipids derived from *Psychrobacter* sp. (▲), *Methanosarcina barkeri* (Δ), *Escherichia coli* (○), *Bacillus stearothermophilus* (■), *Thermotoga maritima* (●), and *Sulfolobus acidocaldarius* (◇). From [27] with permission.

variety of bacterial and archaeal species has revealed that at increasing temperatures, k_{H^+} dramatically increases at a different temperature for each of the liposomes (Fig. 3). To some extent the increase in k_{H^+} correlates with the growth temperature of the organism from which the lipid was isolated. The higher the growth temperature of the organisms from which the lipids were extracted, the higher the temperature is at which a certain proton permeability of the liposomes is reached. Liposomes prepared from lipids derived from the psychrophile *Psychrobacter* sp. were already found to be highly permeable to protons at a low temperature. At around 30°C, the k_{H^+} of the liposomes of this bacterium is at least five-fold higher as compared to that for mesophilic organisms. The mesophilic *M. barkeri* and *E. coli* exhibit a similar temperature dependency of k_{H^+}, whereas the k_{H^+} values for the moderate thermophile *B. stearothermophilus* are hardly distinguishable from that of the mesophiles. According to this study, the membranes of the latter organisms already exhibit a rather high proton permeability at the growth temperature. In order to maintain a viable $\Delta\tilde{\mu}_{H^+}$, *B. stearothermophilus* exhibits an extraordinary high respiration rate that serves to expel protons into the external medium. Consequently, this organism has to invest an increased share of its metabolic energy into the maintenance of a $\Delta\tilde{\mu}_{H^+}$ [28] and to remain viable [29,30]. Liposomes derived from the extreme thermophiles *S. acidocaldarius* and *Picrophilus oshimae* (van de Vossenberg, unpublished results) are much more proton resistant at their respective growth temperatures as compared to the others. The basal proton permeability of these membranes at the respective growth temperatures will therefore be low, allowing efficient proton-linked energy transduction. The activation energy of the proton permeability, $\Delta G'_{H^+}$, ranged from 40 to 55 kJ mol^{-1}, suggesting that even though the liposomes have a vastly different lipid composition, the mechanism of proton permeation is likely to be the same (See following section). However, the temperature range wherein the proton permeability varies is unique for each of the liposomes and correlates more or less with the growth temperature of the organism from which the lipids were isolated (Fig. 3). This has been taken as an indication that an increased proton permeability of the membrane may restrict the ability of an organism to grow at elevated temperatures.

In contrast to protons, a correlation between the growth temperature and permeability has not been observed for sodium ions. Rather, the lipid composition of the membrane has only a minor effect on the membrane permeability for sodium ions (k_{Na^+}, first order rate constant for sodium ion permeability) (Fig. 4). The rate of sodium permeation is only dictated by the temperature and not by the membrane lipid composition. This is consistent with the notion that protons and sodium ions permeate the membrane by distinct mechanisms (See following section). The

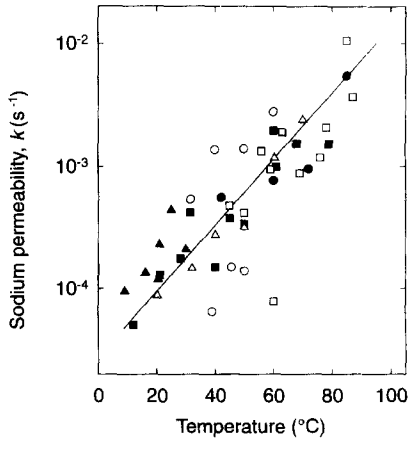

Fig. 4. Temperature dependency of the first order rate constant for the sodium permeability (k_{Na^+}) of liposomes derived from various bacteria and archaea. Further details are as in the legend of Fig. 3. From [27] with permission.

activation energy of the sodium ion permeability, $\Delta G'_{Na^+}$, is about 47 kJ mol^{-1}, and thus is in the same order as found for the proton permeability. However, the permeability of the membrane for sodium ions (k_{Na^+}) is several orders lower than for protons (k_{H^+}). The net flux of sodium ions or protons, however, depends on the (electro-)chemical gradients of these molecules and their absolute concentrations in the external medium and the cytosol.

4. Mechanisms of proton and sodium permeation

Thus far, the molecular mechanism underlying the striking difference between the transport rates of protons on the one hand, and other ions, such as sodium ions, on the other hand, is not well understood. Various models have been proposed, but none of these models sofar accurately describes the permeation processes as they are observed experimentally. Sodium ion (and likely that of other cations) permeation is best described by the 'solubility-diffusion model' (Fig. 5A) [31,32] in which the transport rate of the permeant (J) is proportional to the product of the permeability coefficient, P (in cm/s), the surface area of the membrane, A (in cm^2), and the concentrations of solute, c, in the aqueous phases at the inner and outer sides of the membrane, i.e., c_i^{aq} and c_0^{aq}, respectively. For an uncharged solute the passive flux is described by Eq. (1):

$$J = \frac{KD}{l} A(c_i^{aq} - c_0^{aq}) = PA(c_i^{aq} - c_0^{aq}) = PA\Delta c \quad (1)$$

The permeability coefficient of the membrane, P, is proportional to the diffusion coefficient, D (in cm^2/s) divided by the thickness of the membrane (l). Experimentally, the transport rate of a solute across the membrane can be calculated from the decay times of the concentration gradients, or from conductance measurements. If the driving force is a concentration gradient Δc, the permeability coefficient, P, can be calculated as follows:

$$P = \frac{J}{A\Delta c} \quad (2)$$

P is directly proportional to the rate constants k used to analyse the proton and sodium ion permeability of the membrane as shown in Figs. 3 and 4.

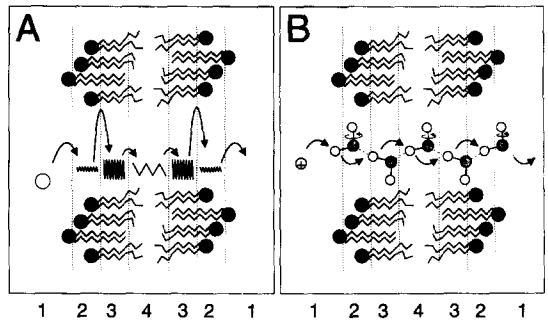

Fig. 5. Schematic representation of the membrane permeation of sodium ions (A) and protons (B). Sodium permeation is depicted according to the solubility-diffusion model through an inhomogeneous phospholipid membrane. The arrows in (A) indicate the solubility steps, and springs indicate the diffusion steps. A high arrow represents a large free energy difference. A stretched spring represents large diffusive jumps. Proton permeation is shown according to the transient hydrogen bonded chain model that assumes the transient formation of strands of water molecules that are connected through the membrane to the opposing water layer. Protons are rapidly transported along this water wire via turning defects of the water molecules or via a hopping mechanism. The inhomogeneous nature of the membrane is indicated by the four regions. Region No. 1 presents the perturbed water in the vicinity of the phospholipid head groups with properties that resemble that of bulk water. Region No. 2 represents the water that is bound to the lipid headgroups. The increased inter hydrogen bonding between water molecules creates a diffusional barrier for hydrated ions. Region No. 3 is nearly free of water molecules, and due to the ordering of the lipid acyl chains behaves as a fluid with high viscosity. In region No. 4, the lipid density is lower than in region No. 3 due to disordering of the acyl chains thereby creating free volume pockets large enough to accommodate water molecules. The diffusion process in this region is characteristic for diffusion in liquids with low viscosity.

Similarly, in the case of a transmembrane electrical potential ($\Delta\psi$), the conductance of a charged solute is obtained by:

$$G = \frac{I}{\Delta\psi} = zF\frac{J}{\Delta\psi} \quad (3)$$

where I is the current density carried by the ions of charge of z electron units. Both type of measurements are related to each other via:

$$P = \frac{RT}{cz^2F^2} G \quad (4)$$

in which F denotes Faraday's constant.

Cations permeate the membrane as hydrated ions [33]. However, in the case of sodium ions, the experimentally determined rates are several orders of

magnitude higher than predicted by the solubility-diffusion model for a hydrated ion [34]. To account for this discrepancy, various adaptations to this model have been proposed amongst others transient membrane defects that would facilitate the solubility step [32,34].

Although the transport rates of ions are reasonably understood with the solubility-diffusion model, in the case of protons the model is not sufficient. Experiments with planar lipid membranes have shown that the resistance of a lipid membrane to ionic conductance is very high. The fluxes of monovalent ions are around 10^{-16} mol cm^{-2} s^{-1}. For proton transport, values of around 10^{-15} mol cm^{-2} s^{-1} have been found [35]. However, the driving concentration differences in the case of ions are in the order of 0.1 M, whereas for proton transport they are only near 0.1 μM. This implies a permeability coefficient for ion transport in the order of 10^{-12} cm s^{-1} vs. 10^{-4} cm s^{-1} for proton transport, a difference of eight orders of magnitude. Typically reported permeability values for proton transport are 10^{-4}–10^{-10} cm s^{-1}, corresponding to conductances of 10^{-7}–10^{-13} S cm^{-2} [35–37] The values provided in Fig. 3 correspond to permeability coefficient of 10^{-9}–10^{-10} cm s^{-1} depending on the temperature. The anomaly of the proton transport mechanism is underlined by observations that the conductance is only slightly dependent on the proton concentration (pH); the conductance increases only 10-fold over a pH range of 1–11 [36]. For other ion transport processes, the conductance increases linearly with the concentration. Furthermore, hydroxide transport is as fast as proton transport implying that both processes are similar [35].

The coupling between proton and water transport is less clear [32,34,38–40]. Experiments on membranes with varying lipid composition indicate no coupling, while temperature dependent measurements indicate similar behaviour for proton and water transport. Both transport processes show no maximum at the main phase transition of the lipids. The experimental evidence sofar suggests an anomalous permeation process for protons across lipid membranes. The most convincing proposal so far is that a water pore might be involved, across which the protons can permeate rapidly via a wire-like conductance mechanism. 'The transient hydrogen bonded chain model' (Fig. 5B) proposed by Nagle [39] assumes that due to thermal fluctuations, a strand of water molecules can connect through the membrane to the opposing water layer, thus forming a water pore. When this happens, protons can be transported very fast by a combined mechanism (through ionic and turning defects), similar to that of proton mobility in ice. In this mechanism, first an ionic defect is transported, via a hopping mechanism, followed by the transport of a turning defect. After the second step, another proton could be transported by the same mechanism. Molecular dynamic simulations indicate that the life-time of such water-wires is very short, i.e., only a few picoseconds, but the predicted rate constants that emerge from this model are in the correct order of magnitude [32]. Unfortunately, the experimental data does not provide clear answers. However, if water pores exist, they are expected to be only transient, and therefore hard to detect.

High proton permeation rates have also been attributed to weakly acidic contaminants, which act as proton carriers. Lipid hydrolysis and oxidation are considered to be possible origins for weak-acid protonophores. According to the 'weak acid model' [38], the anomalous high permeation rate of protons across lipid bilayer membranes would be mainly an experimental artifact, and not an intrinsic property of the bilayer itself. Although experimental data clearly shows that protonophores indeed are a possible transport mechanism for protons, the difference of six to eight orders of magnitude between proton and other cation permeabilities can not be accounted for. At most, it would account for one to two orders of magnitude. Furthermore, the pH dependence of protonophores is considerably different from the pH dependence of proton conductance [32]. Therefore an additional mechanism for proton transport is required. In addition, the striking correlation between the proton permeability and the growth temperature of an organism (Fig. 3), makes contaminating weak acids less likely as major determinants of the proton permeability.

5. Physiological consequences of proton permeation for extremophiles

Some thermophilic bacteria, like *Clostridium fervidus*, exclusively use Na^+ as an energy-transducing

coupling ion [41]. *C. fervidus* is, however, unable to regulate its internal pH and its growth is therefore confined to a very narrow pH range around neutrality [41]. Since *C. fervidus* lacks a proton cycling system, energy transduction solely depends on sodium cycling [42]. This energetic restriction isolates this organism to a highly specialized nice [43]. Clearly, such a specialization would not be profitable to acidophilic and alkaliphilic organisms. These bacteria require at any rate, a cytoplasmic membrane that is equipped with a low proton (or hydroxyl ion) permeability in order to maintain their intracellular pH as depicted in Fig. 2. The group of thermophilic acidophiles and alkaliphiles require further attention in this respect, and are discussed in this section.

For thermophilic alkaliphiles the increase in proton permeability with increasing temperature may be especially pressing as these organisms have to maintain an intracellular pH that is lower than the external pH [20]. This usually involves a Na^+/H^+ antiporter that allows the influx of protons in an electrogenic manner with the concomitant expulsion of sodium ions. In the cases studied sofar, mesophilic aerobic alkaliphiles use protons for energy transduction. To account for the increased proton-permeability at higher temperatures, aerobic thermoalkaliphiles will have to dramatically increase the respiration-linked proton-pumping activity to assure the maintenance of a viable $\Delta\tilde{\mu}_{H^+}$ and intracellular pH. Some aerobic thermoalkaliphiles, however, depend on sodium ions for growth [B. Grant, personal communications]. The mechanism of energy transduction in these organisms has not yet been studied, nor is it known how they maintain their intracellular pH. At any case, one has to assume that the membrane of these organisms is sufficiently impermeable to allow pH homeostasis. In contrast to aerobic thermoalkaliphiles that are presumably equipped with high-fuelled respiratory chains [20], anaerobic counterparts have to rely on their membrane-bound proton or sodium-linked ATPases for the generation of a $\Delta\tilde{\mu}_{H^+(Na^+)}$. Proton-linked energy transduction would require a membrane that is highly impermeable to protons. Sodium-linked energy transduction, on the other hand, raises the problem as to how these organisms maintain their intracellular pH. In a recent study, the energy transducing mechanisms was studied in isolated membranes of *Thermoalkalibacter bogoriae*, a Gram-positive, spore forming anaerobic thermoalkaliphile that grows optimally at 50°C and an alkaline pH of 9.5 [S. Prowe and G. Anthranikian, personal communications]. Energy transduction in this organism was found to be dependent on Na^+ cycling [S. Prowe, J.L.C.M. van de Vossenberg, A.J.M. Driessen, G. Anthranikian, and W.N. Konings, unpublished data]. Therefore, the question can now be addressed: how does this organism as a representative of the poorly studied anaerobic thermoalkaliphiles maintain its intracellular pH. At pH 10–11, alkaliphiles usually maintain an intracellular pH that is about 2 pH units lower than the pH of the suspending medium (Fig. 2). Since *T. bogoriae* primarily relies on a Na^+-translocating ATPase for the generation of a sodium gradient across the membrane, it is difficult to image how a Na^+/H^+ antiporter would contribute to pH homeostasis. The possibility exists that in these organisms the internal pH is regulated by a K^+/H^+ antiporter which would allow the influx of H^+ at the expensive of K^+ expulsion. Alternatively, these organisms may rely on the activity of a pH-regulated H^+-channel that would facilitate the $\Delta\psi$-driven extrusion of hydroxyl-ions, while the intracellular formation of acids may contribute to pH homeostasis. Obviously, the mechanism by which these bacteria regulate their intracellular pH is an important facet of the bioenergetics of anaerobic thermoalkaliphiles that needs to be studied.

Acidophiles are forced to maintain a steep proton gradient across the membrane [44]. This can only be realised with a specific membrane lipid composition. An extreme case of adaptation in this respect is found in the thermoacidophilic Archaea like *S. acidocaldarius* or *P. oshimae*. These cells maintain their internal pH near to neutrality, i.e., pH 6.0–6.5, by an efficient respiratory chain that expels the protons from the cytosol [45]. The extraordinary lipid composition of the cytoplasmic membranes of *S. acidocaldarius* renders the membrane highly resistant to proton permeation even at the higher temperatures [26,27]. In addition, proton influx is counteracted by a reversed $\Delta\psi$, i.e., inside positive [46]. Energy transduction in this organism is proton-coupled. Membranes of *S. acidocaldarius* contains branched (isoprenoid) tetraether chains. These isoprenoid chains are unable to form hydrogen-bonding

chains of water between the phytanyl chains of the membrane lipids, and this phenomenon may contribute to the higher proton permeation resistance of these membrane.

An interesting example is the anaerobic acidophile *Sarcina ventriculi*. This organism is reported to grow over the extraordinary pH range of 2.0–9.0, with an intracellular pH of about 4.3 at an external pH of 3.0 [47]. Vital cellular processes in this anaerobe are apparently not inactivated by the low intracellular pH. It would be of interest to determine how anaerobic thermoacidophiles, insofar they exist, cope with this problem.

6. Concluding remarks

In conclusion, thermophiles have developed different mechanism to cope with the increased permeability of their membrane to protons at higher temperatures. They may sustain the electrochemical gradient of protons by increasing the rate of proton pumping to compensate for the increased permeability at higher temperature. Consequently, an increased fraction of the metabolic energy is used for maintenance. Alternatively, they may alter their membrane composition such that the membrane becomes less permeable to ions. Possibly as a last resort, cells may entirely change their energy transducing mechanism by coupling these processes to an ion that is less permeable than protons, such as sodium ions [48].

Acknowledgements

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